

FEB. 6. 2006 8:30PM ENZO BIOCHEM

NO. 7993 P. 132

Stavrianopoulos et al., Serial No. 08/486,070 (Filed June 7, 1995)
Exhibit 12 [Fifth Supplemental IDS -- February 6, 2006]

EXHIBIT 12

Enz-7(P)(C3)

Cytochemistry (cytochemistry)

This is a discipline derived from cytology* which studies the relationship with the structure and functions of the cells through studying the localization of various substances existing in the cells by checking the cells themselves or fixed pieces of the cells. Research techniques used for detection and determinate quantity of substances in the cells are almost same as those of histology. Optical microscopes or electronic microscopes are used. Various techniques such as microscopic spectrophotometry including ultraviolet absorption, fluorescence microscopic technique, detection of specific biological matters and enzymes through chemical reaction or enzyme reaction, etc. are used depending on the objective. This discipline emerged by applying knowledge about biological materials obtained from biochemistry, which rapidly developed at that time, while development of classical cytology had already ended by the beginning of the 20th century; however, cell biology that was newly derived from cytology as cell fractionation was established and expanded as a new large discipline for cell research.

Histochemistry (histochemistry)

This is a field of animal morphology and its purpose is to precisely understand biological activity, enzyme activity and minute localization of a group of functional atoms; to understand the field of reaction and function and intracellular compartment; to understand the dynamics of subcellular organelle at a minute structural level. Recently there is almost no boundary with cytochemistry. It originated from a study of distributions *in situ* utilizing chemical reaction and enzyme reaction to produce an insoluble colored substance, iodine reaction of starch was used in the mid-19th century. In the 20th century, it was developed by J. Bruchet, T.O. Casperson, D. Glick, G. Gomori, Hideo Takamatsu, A. G. E. Pearse, L. Lison and so on. Sometimes cytochemistry is separately classified from optical microscopic cytochemistry and electronic microscopic cytochemistry from the viewpoint of analytical technique, however, it can be classified under quantitative cytochemistry (K. U. Landerström-Lang) and fractional method (R. R. Bensley) in addition to microscopic cytochemistry. For identification of a substance, microscopic spectrophotometry, fluorescence antibody technique, ferritin antibody method, and autoradiography in addition to specific color reaction are used.

Fluorescent antibody technique (fluorescent antibody technique, munofluorescence technique)

This is a detection method used under fluorescence microscopes by combining antigenic substances such as structural proteins and microorganisms with fluorescent antibodies. [illegible] isothiocyanate (FITC) is often used as fluorescent labeling, however, tetramethyl rhodamine isothiocyanate (RITC) is also used. Both are different in fluorescent wavelength or color, therefore, they can be used to simultaneously detect two antigenic substances in the same sample by double coloring. As a method to directly label the antibody for antigen to be detected, there is a method to label the second antibody of the antibody of the agonist (indirect method) and another method to combine the second fluorescence [illegible] via catalyst. Recently a method of using enzyme labeling instead of fluorescence labeling (→ enzyme antibody) has come to be used, and [text cut off] immunoassay using fluorescence labeling (fluorescent immunoassay*) has been developed as well. (→ indirect fluorescence antibody technique)

Enzyme-labeled antibody technique, immunoenzymatic technique (enzyme-labeled antibody technique, immunoenzymatic technique)

This is a method to detect antigenic substances such as structural proteins and microorganisms by using the antibody labeled by enzyme. Peroxidase is mostly used as a labeling enzyme, however, glucose oxidase, tyrosinase, acid phosphatase, and alkaline phosphatase can be used and multiple colorizations are possible. This technique can be used not only under optical microscopic detection but also electronic microscopic detection. Similarly to the fluorescent antibody technique* and ferritin antibody technique, both direct method and indirect method can be used.